Bioorganic & Medicinal Chemistry Letters 19 (2009) 6797–6800

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Heterocyclic 1,7-disubstituted indole sulfonamides are potent and selective human EP_3 receptor antagonists

Georgeta Hategan [a,†], Alexandre M. Polozov [a,†], Wayne Zeller [a], Hua Cao [a], Rama K. Mishra [a], Alex S. Kiselyov [a], Jose Ramirez [b], Guðrún Halldorsdottir [b], Þorkell Andrésson [b], Mark E. Gurney [b], Jasbir Singh [a,*]

[a] Medicinal Chemistry Department, deCODE Chemistry, Inc., 2501 Davey Road, Woodridge, IL 60517, United States
[b] deCODE Genetics, Inc., Reykjavik, Iceland

ARTICLE INFO

Article history:
Received 31 May 2009
Revised 22 September 2009
Accepted 22 September 2009
Available online 27 September 2009

Keywords:
Prostanoid receptor antagonists
EP_3 receptor antagonists
Peri-substituted indoles
Acylsulphonamide
Animoazoles
3-Amino-isoxazoles

ABSTRACT

We have developed a pharmacophore model for the EP_3 receptor antagonists based on its endogenous ligand PGE_2. This ligand-based design yielded a series of novel peri-substituted [4.3.0] bicyclic aromatics featuring 1-alklyaryl 7-heterocyclic sulfonamide substituents. The synthesized molecules are potent antagonists of human EP_3 receptor in vitro and show inhibition of rat platelets aggregation. Optimized derivatives display high selectivity over IP, FP, and other EP receptor panels.

Prostanoids are of key importance in the regulation of platelet function. Their activities are mediated via a family of G-protein coupled receptors (GPCRs) and are tightly regulated. Prostaglandin E_2 (PGE_2) binds preferentially to the E prostanoid family of receptors, of which there are four subtypes, referred to as EP_{1-4}.[1] Furthermore, studies in EP_3 knock-out (KO) mice confirmed that the stimulatory effects of PGE_2 on platelet aggregation are exerted specifically through the EP_3 receptor.[2] Additionally, PGE_2 has been shown to be critical for ion transport,[3] smooth muscle contraction of the GI tract, acid secretion, uterine contraction during fertilization and implantation,[4] fever generation, and hyperalgesia.[5] Potent and specific antagonists of EP_3 receptor are anticipated to feature anti-thrombotic effects without increasing the risk of bleeding.[6]

We have reported previously that 1,7-disubstituted indole analogs **A** (Fig. 1) were potent, isoform selective human EP_3 receptor antagonists. They also exhibited sound activity in the functional platelet aggregation assay.[6] The acylsulfonamide group in **A** has been shown to be important for hEP_3 receptor affinity. Namely, replacement of the acylsulfonamide moiety ($CONHSO_2$) with either an amide (CONH) or sulfonamide ($NHSO_2$) groups resulted in loss of hEP_3 receptor affinity. Notably, in our refined pharmacophore model,[7] the acylsulfonamide pharmacophore is represented as a contiguous hydrogen-bond 'acceptor–donor–acceptor' array with the carbonyl oxygen being represented as a hydrogen-bond acceptor (HBA). Thus, any proposed replacement of –C═C–C═O functionality would require a proximal heteroatom capable of serving as a surrogate carbonyl oxygen.

We therefore introduced the isosteric replacement of –C═C–C═O– functionality in **A** with a heterocycle, exemplified by compound **B**, which contains the requisite HBA, Y, in the structure (Fig. 1). The main goals of this work were to (a) demonstrate that heterocyclic replacement of α,β-unsaturated amide provides novel chemotypes exhibiting EP_3 receptor antagonist activity, (b) display

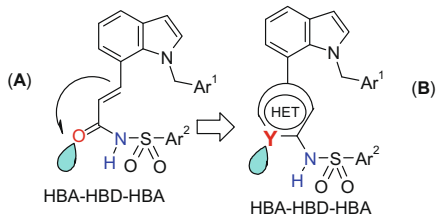


Figure 1. Proposed isosteric replacement of α,β-unsaturated amide with heterocycles.

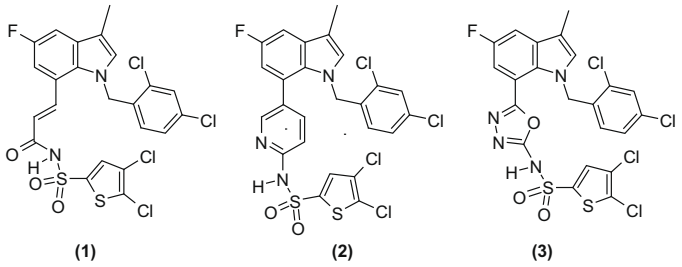
* Corresponding author. Tel.: +1 630 783 4915; fax: +1 630 783 4646.
E-mail address: jsingh@decode.com (J. Singh).
† These authors contributed equally.

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.09.084

prostanoid receptor panel selectivity of these analogs, and (c) assess in vitro metabolic stability of the resultant molecules.

The 5-fluoro-3-methyl indole was selected as the core template for these studies. This core has been previously[6] shown by our group to feature good metabolic stability. Similar to the acyclic derivative **1**, both six- and five-membered bioisosteric replacements, exemplified by 2-pyridyl derived analog **2** and the oxadiazole analog **3**, were shown to be highly potent in the hEP_3 radioligand displacement assay (Fig. 2). Compound **3** also featured much lower plasma protein binding compared to **2**. Driven by this observation, our further SAR studies were centered around five-membered heterocycles.[8] This Letter describes the EP_3 activity, isoform selectivity, and functional activity of C7 indole analogs substituted with azoles as isosteric replacement for the α,β-unsaturated amide.

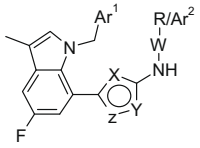
Replacement of the 1,3,4-oxadiazole group in **3** with the isomeric 1,2,4-oxadiazole **4** or isoxazoles **5** and **6** generally provided equipotent analogs in the ^{3}H-PGE_2 radioligand displacement assay



	(1)	(2)	(3)
hEP_3 IC_{50}	4.6 nM	2 nM	10 nM
Fold-shift with 10% human serum	1.3 X	217x	27x

Figure 2. Initial heterocyclic analogs.

Table 1
hEP_3 activity for 7-heterocylic 1,7-disubstituted indole sulfonamides



Compd	X	Y	Z	W	R/Ar^2	Ar^1	hEP_3 NB μM	FS-HS[a]
2	CH–CH	N	CH	SO_2	4,5-Cl2 Thiophene	2,4-Cl2 Benzyl	0.002	217.0
3	O	N	N	SO_2	4,5-Cl2 Thiophene	2,4-Cl2 Benzyl	0.010	27.0
4	N	N	O	SO_2	4,5-Cl2 Thiophene	2,4-Cl2 Benzyl	0.024	20.9
5	CH	N	O	SO_2	4,5-Cl2 Thiophene	3,4-F2 Benzyl	0.059	24.2
6	CH	N	O	SO_2	4,5-Cl2 Thiophene	2,4-Cl2 Benzyl	0.005	67.0
7	CH	N	O	SO_2	4,5-Cl2 Thiophene	2-Naphthyl	0.001	3.6
8	CH	N	N–CH_3	SO_2	4,5-Cl2 Thiophene	3,4-F2 Benzyl	1.978	1.0
9	CH	N	O	SO_2	2,4,5-F3 Phenyl	2-Naphthyl	0.008	2.3
10	CH	N	O	SO_2	2,4,5-F3 Phenyl	2,4-Cl2 Benzyl	0.003	3.3
11	O	N	N	SO_2	2,4,5-F3 Phenyl	2,4-Cl2 Benzyl	0.002	22.0
12	CH	N	O	SO_2	3,4-Cl2 Phenyl	2-Naphthyl	0.011	4.9
13	CH	N	O	SO_2	3,4-Cl2 Phenyl	2,4-Cl2 Benzyl	0.001	18.0
14	O	N	N	SO_2	3,4-Cl2 Phenyl	2,4-Cl2 Benzyl	0.004	3.5
15	CH	N	O	SO_2	3,4-F2 Phenyl	2,4-Cl2 Benzyl	0.001	12
16	CH	N	O	SO_2	3,4-F2 Phenyl	2-Naphthyl	0.001	14.6
17	O	N	N	SO_2	3,4-F2 Phenyl	2,4-Cl2 Benzyl	0.020	2.4
18	CH	N	O	SO_2	2,4,5-F3 Phenyl	3,4-F2 Phenyl	0.012	103.0
19	CH	N	O	SO_2	3,4-F2 Phenyl	3,4-F2 Phenyl	0.044	177.9
20	O	N	N	CO	3,4-F2 Phenyl	2,4-Cl2 Benzyl	0.664	19.6
21	O	N	N	CO	2,4-Cl2 Phenyl	2,4-Cl2 Benzyl	1.315	1.0
22	O	N	N	CO	2,4-F2 Phenyl	2,4-Cl2 Benzyl	1.020	NA
23	O	N	N	CO	2-Furanyl	2,4-Cl2 Benzyl	0.363	NA
24	O	N	N	CO	2,2-Difluoro-benzo[1,3]dioxol-5-yl	2,4-Cl2 Benzyl	0.244	21.4
25	O	N	N	SO_2	CH_3	2,4-Cl2 Benzyl	2.570	199.0
26	CH	O	N	SO_2	CH_3	3,4-F2 Benzyl	17.850	NA
27	O	N	N	SO_2	3,4-F2 Phenyl	3-OMe Phenyl	99% at 1 μM, 35% at 0.1 μM	NA
28	O	N	N	SO_2	4-F Phenyl	3-OMe Phenyl	99% at 10 μM, 62% at 1 μM	NA
29	O	N	N	SO_2	4-F Phenyl	2,3-dihydro-benzo[1,4]dioxin-6-yl	48% at 10 μM, 11% at 1 μM	NA

NB: normal assay buffer.[6]
FS-HS: fold shift in hEP_3 IC_{50} in the presence of 10% human serum.
Compounds **20**–**24** gave only partial displacement of ^{3}H-PGE_2 in the radioligand displacement assay.
Compounds **27**–**29** were evaluated using FLIPR assay.
[a] For selected analogs the percent protein binding was also determined using HSA-HPLC column method,[16] and these data are: compd no. (% bound) **3** (99.8%), **11** (99.4%), **14** (97.2%), and **15** (98.4%).

(Table 1). Notably, the pyrazole derivative **8** (Z = NCH_3) exhibited a 33-fold drop in activity when compared with similar isoxazole molecule **5** (Z = O). We attributed this observation to the presence of the methyl group preventing optimal alignment of the HBA ring heteroatom (Y = N) in **8** at the receptor binding site. Similar to our earlier SAR data from the indole series,[6,9] molecules containing relatively lipophilic, halogenated aromatic groups at Ar^1 and/or Ar^2, provided good activity. This trend was observed irrespective of the nature of the heterocycle. Compounds **9**–**17** also showed low to modest plasma protein binding (PPB), as indicated by the IC_{50} fold-shift in the presence of 10% human serum (Table 1). Simultaneous presence of fluorinated Ar^1 and Ar^2 substituents in the molecule led to good activity against the hEP_3 receptor. Unfortunately, the respective derivatives also displayed a high propensity for PPB (Table 1, **18** and **19**). The contiguous hydrogen bond triad acceptor–donor–acceptor as represented by the heterocyclic sulfonamides could not be supplanted by the analogous amides. Specifically, replacement of SO_2 functionality with CO led to over 30-fold drop in activity for the amide (compare **17** with **20**). This observation was further confirmed for the amides **22**–**24** featuring consistently higher IC_{50} value compared to the respective sulfonamides.[10] Moreover, the amide analogs provided only partial displacement of ^{3}H-PGE_2 in the hEP_3 radioligand displacement assay. In comparison, sulfonamides showed full displacement of the radioligand whereas the amide **21** failed to show full displacement even at the highest assay concentration (ca. 20 μM). Figure 3 shows representative examples of the dose–response curves for the sulfonamide and amide analogs, (**15**) and (**21**), respectively.

Even though the heterocyclic sulfonamides generally provided very potent analogs, the nature of the group attached to the SO_2 of sulfonamide also impacted EP_3 activity (IC_{50}) of the resulting molecules. Similar to our earlier observations, halogenated aromatic phenyl or heterocyclic Ar^2 groups consistently provided the best hEP_3 activity and displayed low plasma protein binding.[11,12] In evaluating the SAR for the Ar^1 group, we focused on the replacement of lipophilic 2-naphthyl and 2,4-dichlorophenyl substituents. Consistent with the SAR in the acyclic α,β-unsatu-

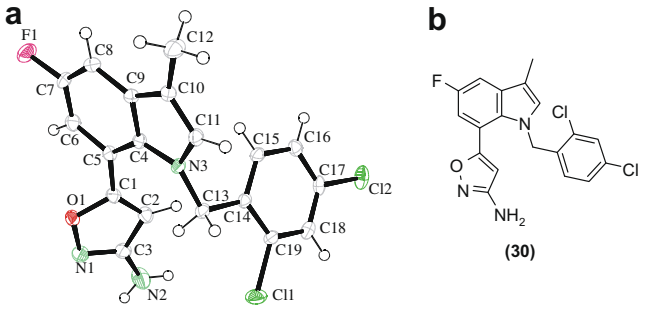


Figure 4. (a) ORTEP plot with thermal ellipsoids at the 50% probability level. (b) Chemical structure of the compounds used for X-ray analysis.

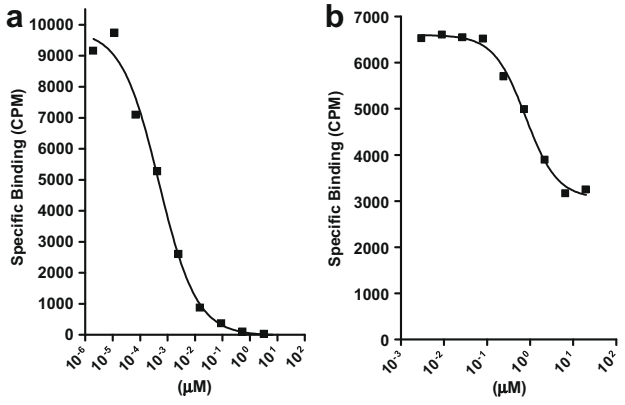


Figure 3. (a) Sulfonamide analog (**15**) showing full displacement and (b) amide analog (**21**) showing partial displacement of ^{3}H-PGE_2 in the in vitro binding assay.

Table 2
Metabolic stability and rat platelet aggregation assay results[a]

Compd No.	Metabolic stability[b]		Rat PAA[c] IC_{50} (nM)
	Rat	Human	
3	46.4	62.7	ND
6	57.8	46.8	1042
7	ND	ND	3242
14	44.8	72.1	2859
15[d]	51.0	46.8	3850
16	9.9	ND	ND
17	42.5	ND	2714

[a] See Ref. 6 for experimental details.
[b] Percent of parent compound remaining after 30-min incubation with the respective liver microsomal preparations, as determined by LC/MS/MS.
[c] Platelet aggregation, induced by PGE_2 and collagen as coaggregant, using rat platelet-rich plasma containing 20% serum.
[d] Compound **15** gave an IC_{50} of 785 nM when platelet aggregation experiments was performed using human platelet-rich plasma.

Table 3
Activity in prostanoid receptor panel[a,b]

Compd no.	EP1 (μM)			EP4 (μM)			FP (μM)			TP (μM)		
	10 μM (%)	1 μM (%)	0.1 (%)	10 μM (%)	1 μM (%)	0.1 (%)	10 μM (%)	1 μM (%)	0.1 (%)	10 μM (%)	1 μM (%)	0.1 (%)
4	100	74	5	25	−21	−21	30	−2	−4	98	5	2
5	52	−5	−8	18	11	9	31	18	8	11	−3	3
7	80	0	−10	10	−9	−9	7	−4	−7	7	−7	−12
9	100	29	−2	9	−17	−17	19	3	−3	19	−5	−9
11	99	94	25	84	15	9	64	21	5	99	11	3
12	16	−7	2	3	−15	−29	−6	−8	−3	−13	−8	9
14	100	16	−2	−7	−22	−26	−1	−2	−15	56	0	3
16	62	9	−3	−4	−17	−25	6	0	−9	12	−2	3
17	100	30	−11	32	26	13	41	26	22	89	9	−5
18	99	77	5	38	−3	−8	99	−11	3	70	3	5
19	99	11	−9	18	17	18	21	16	15	17	0	3
20	96	10	−7	62	14	9	66	15	3	64	−1	11

[a] The data shown in table is from FLIPR assay, which were performed at MDS Pharma; data shown is an average of $n = 2$.
[b] Compound **15** was evaluated by radioligand displacement assays, which provided the following IC_{50} data: hEP_1 >20 μM, hEP_2 = 19.8 μM, hEP_4 = 18.6 μM, hFP = 0.6 μM, hIP >20 μM.

rated amide series (**1**),[13] more hydrophilic moieties (e.g., **27**–**29**) yielded poor hEP_3 receptor activity.[14]

The structure of the 3-aminoisoxazole derivative (**30**) was determined using single crystal X-ray analysis (Fig. 4). The intermediate **30** was utilized to synthesize analogs **6**, **10**, **13**, and **15** confirming Y = N provides potent isoxazole-3-sulfonamide analogs.

Selected analogs showing good IC_{50} for hEP_3 receptor and low plasma protein binding were further examined for their in vitro microsomal stability using rat and human liver microsome preparations. As shown in Table 2, with the exception of the 2-naphthyl analog (**16**), all analogs tested displayed reasonable microsomal stability.

Several compounds were further evaluated for their activity in a panel of prostanoid receptors. As shown in Table 3, molecules **4**, **11**, **15**, and **18** are projected to have sub-micromolar IC_{50} for EP_1. The other derivatives examined afforded low affinity for the prostanoid receptors, including EP_1, EP_4, FP, and DP isoforms.

Considering this encouraging in vitro data, several prioritized analogs were further evaluated in the secondary rat platelet aggregation assay (PAA), where aggregation was initiated with PGE_2 in the presence of collagen. Isoxazole **6** showed a $\sim 2\times$ better activity than the two oxadiazoles **14** and **17**, while the isoxazole **15** showed slightly lower activity (Table 2). The reason for variance in the in vitro receptor assay (Table 1) and functional assay (Table 2) IC_{50} values would appear to be due to differences in sensitivity for human versus rodent receptors for these analogs. This notion was further supported by the fact that compound **15** gave a five-fold better inhibition when evaluated using human platelet-rich plasma.[6,15] Compound **15** along with several representative molecules from this class are being further evaluated in the panel of follow-up assays in order to select an optimized lead candidate and these data will be reported in the future.

In conclusion, we have described SAR of 1,7-disubstituted indoles featuring isosteric replacement of the C7 α,β-unsaturated amide group with five-membered heterocycles. This new series exhibits high affinity for the human EP_3 receptor, sound selectivity across a panel of prostanoid receptors, good microsomal stability, and favorable plasma binding properties. In addition, optimized derivatives were functionally active in the rat platelet aggregation assay.

Acknowledgments

We wish to acknowledge Dr. Jun Zhang and Mr. Emmanuel Onua for conducting metabolic stability studies.

Supplementary data

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 734099. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk]. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.084.

References and notes

1. (a) Abramovitz, M.; Adam, A.; Boie, Y.; Godbout, C.; Lamontagne, S.; Rochette, C.; Sawyer, N.; Tremblay, N. M.; Belley, M.; Gallant, M.; Dufresne, C.; Gareau, Y.; Ruel, R.; Juteau, H.; Labelle, M. *Biochim. Biophys. Acta* **2000**, *1483*, 285; (b) Kiryiama, A.; Ushukubi, K.; Kobayashi, T.; Hirata, M.; Sugimoto, Y.; Narumiya, S. *Br. J. Pharmacol.* **1997**, *122*, 217.
2. Fabre, J.; Nguyen, M.; Athirakul, K.; Coggins, K.; McNeish, J. D.; Austin, S.; Parise, L. K.; FitzGerald, G. A.; Coffman, T. M.; Koller, B. H. *J. Clin. Invest.* **2001**, *107*, 603.
3. Garcia-erez, A.; Smith, W. *J. Clin. Invest.* **1984**, *74*, 63.
4. Krall, J. F.; Barrett, J. D.; Jamgotchian, N.; Korenman, S. G. *J. Endocrinol.* **1984**, *102*, 329.
5. (a) Xin, L.; Geller, E. B.; Bastepe, M.; Raffa, R. B.; Mao, G.-F.; Ashby, B.; Adler, M. W. *J. Therm. Biol.* **2000**, *25*, 77; (b) Minami, T.; Nishihara, I.; Ito, S.; Hyodo, M.; Hayaishi, O. *Br. J. Pharmacol.* **1994**, *112*, 735; (c) Reinold, H.; Seifollah, A.; Ulrike, D.; Beate, L.; Heindl, C.; Hamza, M.; Pahl, A.; Brune, K.; Shuh, N.; Muller, U.; Zeilhofer, H. *J. Clin. Invest.* **2005**, *115*, 673.
6. Singh, J.; Zeller, W.; Zhou, N.; Hategen, G.; Mishra, R.; Polozov, A.; Yu, P.; Onua, E.; Zhang, J.; Zembower, D.; Kiselyov, A.; Ramírez, J.; Sigthorsson, G.; Bjornsson, J.; Thorsteinnsdottir, M.; Andrésson, T.; Bjarnadottir, M.; Magnusson, O.; Stefansson, K.; Gurney, M. A. C. S. *Chem. Biol.* **2009**, *4*, 115.
7. We have generated a pharmacophore model for peri-disubstituted bicyclic series of analogs as the EP_3 antagonists. These studies revealed that representation of the acylsulfonamide as an anionic feature provided a poor correlation for predictive versus observed IC_{50} values than when this feature is represented as a neutral species, as shown in Figure 1. The details of the pharmacophore model development will be reported in a forthcoming disclosure.
8. Polozov, A. P.; Hategan, G.; Cao, H.; Zeller, W.; Kiselyov, A. S.; Singh, J. *Tetrahedron Lett.*, submitted for publication.
9. Zhou, N.; Zeller, W.; Keyvan, M.; Krohn, M.; Anderson, H.; Mishra, R.; Zhang, J.; Onua, E.; Ramirez, J.; Palsdottir, G.; Halldorsdottir, G.; Andresson, T.; Gurney, M.; Singh, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 123.
10. The in silico structures of **15** (sulfonamide) and **20** (amide) were generated from the X-ray coordinates of compound **30**. These structures were minimized using MMFF94. These minimized structures were subjected to systematic search sub-routine in SYBYL 6.8. This analysis showed that the sulfonamide triad: HBA–HBD–HBA all project from the same face-of the molecule while the low energy conformation of the corresponding amide does not allow such orientation. The amide conformations that provide such a scenario are ~4 kcal/mol higher than the lowest conformer. This is anticipated considering that the sulfur atom of the sulfonamide is essentially pseudo sp^3 compared with the amide carbonyl being sp^2.
11. The heterocylic sulfonamides showing hEP_3 IC_{50} <100 nM, provided a range of calculate $pK_a = 4–7$.
12. For the pairs of analogs, **3** versus **25** and **5** versus **26**, replacement of aromatic versus methyl at Ar^2 result in calculated ΔpK_a of 1.7 units.
13. Analogs bearing 2, 3, or 4-pyridyl methyl group at the indole nitrogen in the acyclic α,β-unsaturated acyl-sulfonamide series provided hEP3 IC_{50} >2 μM.
14. These compounds were evaluated in a FLIPR assay, which had been shown to provide comparable results to ^3H-PGE_2 radioligand displacement assay.
15. A cinnamic acylsulfonamide EP_3 receptor antagonist compound **6d** reported by Merck displayed IC_{50} of 18 nM and 563 nM for human and mouse EP3 receptors, respectively. For the structure of compound **6d**, see: Juteau, H.; Gareau, Y.; Labelle, M.; Sturino, C.; Sawyer, N.; Tremblay, N.; Lamontagne, S.; Carriere, M.; Denis, D.; Metters, K. *Bioorg. Med. Chem.* **2001**, *9*, 1977.
16. Chromtech immobilized Chiral-HSA column (50 × 3 mm, 5 μm) was used for the assay. The mobile phase A was 50 mM pH 7.4 phosphate buffer solution, while mobile phase B was *i*-PrOH. The flow rate was 0.5 mL/min. From 0 to 2 min, the mobile phase composition was constant 4% *i*-PrOH and 96% phosphate. From 2 to 7 min, the linear gradient from 4% to 30% *i*-PrOH was applied. From 7 to 16 min, the mobile phase composition was constant 30% *i*-PrOH and 70% phosphate. From 16 to 17 min the mobile phase composition was changed to 4% *i*-PrOH and 96% phosphate until the end of the run at 30 min.